Classification and Treatment of Chronic Nonhealing Wounds

Successful Treatment with Autologous Platelet-derived Wound Healing Factors (PDWHF)

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Previous animal data showed that platelets contain growth factors that stimulate capillary endothelial migration (angiogenesis), fibroblast proliferation and migration, and collagen synthesis. This study utilized autologous platelet-derived wound healing factors (PDWHF) to treat 49 patients with chronic nonhealing cutaneous ulcers. Patients were classified on the basis of 20 clinical and wound status parameters to generate a wound severity index. Forty-nine patients—58% diabetic (20% with renal transplants); 16% with trauma, vasculitis, etc.; 14% with decubitus ulcers; and 6% each with venous stasis or arterial insufficiency—with a total of 95 wounds had received conventional wound care for an average of 198 weeks (range: 1–1820 weeks). After informed consent was obtained, patients received autologous PDWHF. Mean 100% healing time for all patients was 10.6 weeks. There was no abnormal tissue formation, keloid, or hypertrophic scarring. A multivariant analysis showed a direct correlation to 100% healing with initial wound size and the initiation of PDWHF therapy. This is the first clinical demonstration that locally acting growth factors promote healing of chronic cutaneous ulcers.

THE BIOCHEMICAL, cellular, and environmental regulation of wound repair involves a complex interaction between serum enzyme cascades, locally acting growth factors, circulating platelets and monocytes, tissue macrophages, fibroblasts, endothelial cells, epidermal cells, and the local cellular microenvironment.[1] Recent advances in the biology of wound healing demonstrate that macrophages and platelets are the predominant regulatory cells in the repair process.[2] Platelets initiate this repair by releasing potent locally acting growth factors. Wound macrophages take over the regulatory role from platelets 24 hours after wounding and continue to produce similar locally acting growth factors until the repair is complete.[3]

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Utilizing the circulating platelet as a source of autologous locally acting growth factors, we tested the efficacy of topically applied platelet-derived wound healing factors (PDWHF) in stimulating the repair of chronic nonhealing cutaneous wounds.

In order to quantitatively categorize and follow the clinical course of repair, a wound severity score was established by assigning a score to various clinical, anatomic, and observed wound and patient variables. The rate of PDWHF-stimulated wound repair and the effect of various measured and observed variables on repair were then analyzed and compared.

Materials and Methods

Patient Selection

Patients with chronic nonhealing cutaneous wounds referred to the Wound Healing and Limb Salvage Clinic at the University of Minnesota were eligible for participation in this study. Authorization from the Institutional Review Board was obtained, and all patients were given informed consent before inclusion in the study. Patients with wounds that possibly contained viable malignant cells were not eligible for PDWHF treatment and were thus excluded.

Wound Severity Score

A wound severity score was devised from clinical, anatomic, and measured wound and patient variables. Scores were arbitrarily assigned and weighted using traditional wound healing clinical experience. These general wound parameters are listed in Table 1. Anatomic considerations such as the presence of exposed bone or tendon, wound location, and the quality of the pedal and posterior tibial

TABLE 1. *Total Wound Score—General Wound Parameters*

	None	Mild	Marked
Periwound erythema	0	2	4
Periwound edema	0	2	4
Wound purulence	0	3	6
Wound fibrin	0	2	4
Limb pitting edema	0	2	4
Limb brawny edema	0	3	6
Wound granulation	4	2	0

pulses (when relative to wound location) were recorded and scored (Table 2). Wounds were measured to determine total wound surface area, depth, and the extent of undermining. The wound surface area was determined by photographing the wound at a fixed magnification using a Kiron 105 mm macro lens (Kiron Corporation, Carson, CA) at the same magnification at each clinic visit. The developed slide was then projected on a computer-aided planimeter and the surface area was determined to the nearest square millimeter. Three measurements were made and the final surface area was the mean of the three measurements. The duration of the wound was determined by patient history. The scores assigned to these various wound measurements are found in Table 3.

The initial and subsequent wound scores were recorded and tabulated at each clinic visit by the two clinical and research registered nurse wound healing specialists. These determinations were periodically checked by the principal investigator.

Wound Care Protocol

Patients entered into the study received complete history and physical exams. The underlying pathology behind the formation of the ulcer was determined and recorded in addition to the infection status of the ulcer at presentation. Superficially infected wounds were treated with topical silver sulfadiazine (Silvadene, Marion Laboratories, Inc., Kansas City, MO) and/or oral antibiotics if indicated. Wounds with invasive infection were treated with silver sulfadiazine and intravenous antibiotics.

At the initial clinic visit, if indicated, the wounds were sharply debrided of all necrotic soft and hard tissue if the patient could tolerate the procedure in the clinic. Wounds with a significant amount of infection were debrided in ambulatory surgery. At each subsequent clinic visit, all wounds were inspected and were debrided of any necrotic material or fibrin that may have developed.

Standard clinical and surgical care was taken to improve skin perfusion in the devitalized area. For example, patients with venous stasis ulcers were treated with compression dressings and leg elevation. Patients with arterial insufficiency due to atherosclerosis or diabetes mellitus were studied by noninvasive vascular assessment, evaluated with arteriography, if indicated, and revascularized if possible.

Wounds were treated with PDWHF when all superficial purulence and necrotic tissue were eliminated and the extremity was revascularized.

Patients were generally treated as outpatients and were examined in the clinic every other week.

Definition of Successful Healing

A wound was classified as healed when the ulcer was completely covered with new epithelium. This was determined visually during the wound evaluation performed in the course of the routine follow-up schedule.

Preparation of PDWHF

PDWHF was prepared from each patient's blood according to previously described methods.[2] Briefly, 60 ml of blood was drawn into a syringe containing 6 ml of anticoagulant citrate dextrose. The blood was immediately put on ice for transport from the clinic to the laboratory. The red and white blood cells were removed by centrifugation (135 × g, 20 minutes at 4 C) to leave a platelet-rich plasma. The remainder of the serum and cells was discarded. A platelet count was done, the platelets were removed from the platelet-rich plasma by centrifugation (750 × g, 10 minutes at 4 C), and the plasma was discarded. The platelets were washed with a buffer solution and subsequently resuspended in buffer at a concentration of 10^9 platelets/ml. The platelets were then released with thrombin (Thrombinar, Armour Pharmaceutical Co., Kankakee, IL) (1 U/ml) to create a supernatant that contained the PDWHF. The remaining spent platelets were removed by centrifugation (950 × g, 5 minutes at 4 C) and discarded.

The PDWHF (10 ml) was then added to a 1 gram jar of microcrystalline collagen (Avitene, Alcon Laboratories, Inc., Fort Worth, TX) to produce a sterile topical salve.

TABLE 2. *Total Wound Score—Anatomic Considerations*

Exposed Bone	Score	Exposed Tendon	Score	Dorsalis Pedis Pulse	Score	Posterior Tibial Pulse	Score
Yes	10	Yes	7	0–1+	5	0–1+	5
No	0	No	0	2+	2	2+	2
				3–4+	0	3–4+	0

TABLE 3. *Total Wound Score—Wound Measurements*

Size (cm^2)	Score	Depth (mm)	Score	Undermining (mm)	Score	Duration	Score
<1	0	<5	0	<2	3	<8 wk	0
1–2	1	5–10	3	2–5	5	8 wk–6 mo	1
2–5	3	10–20	7	>5	8	6 mo–1 yr	2
5–10	6	>20	10			2–3 yr	5
10–30	8					3–5 yr	7
>30	10					5–10 yr	9
						>10 yr	10

One 10 ml aliquot of the salve was used for 1 week and then discarded.

Each preparation of the PDWHF was tested for sterility after preparation. Representative aliquots of the PDWHF were tested for mitogenic potential using a standard assay.[4]

PDWHF Application Protocol

When PDWHF therapy was initiated, the patients were instructed to apply a thin layer of the PDWHF salve to the entire surface of the wound. This was covered with petroleum-impregnated gauze and a sterile gauze dressing. The PDWHF was left in place for 12 hours and then was completely removed by washing with tap water. Silver sulfadiazine was then applied for 12 hours and at the end of that time removed with tap water irrigation. Occasionally, patients used a water jet toothbrush to irrigate their wounds at home.

Statistical Evaluation

All measured parameters of the patients and their individual wounds were recorded and computerized. The data were analyzed utilizing Version 9 of the Statistical Package for the Social Sciences (SPSS) (University of Chicago, Chicago, IL). Continuous data comparisons (T100 *vs.* size score, etc.) were first assigned F values, and, subsequently, two-tailed probabilities were derived. Direct Pearson correlation coefficients were calculated for the suspected linear correlates between healing times and pertinent wound characteristics. Overall mean healing time equations for the different temporal categories based on our wound severity index were obtained utilizing a multiple regression analysis. Finally, the Student's t-test was applied when comparing mean healing times in the T100 *versus* T2 and diagnosis categories.

TABLE 4. *Tabulation of Number of Patients and Wounds in Each Diagnosis Category**

	Initial Number	Lost to Follow-up	Surgical Cure	Final Analysis
Decubitus ulcer	7–15	0–0	1–4	6–11
Diabetes	19–35	0–0	2–2	17–33
Transplanted diabetic	9–9	2–5	0–0	7–4
Arterial insufficiency	3–4	0–0	0–0	3–4
Venous stasis	3–9	1–3	0–0	2–6
Other	8–23	1–3	1–7	6–13
Total	49–95	4–11	4–13	41–71

* Those deleted from the study and the final patient profile are listed.
First entry = # of patients.
Second entry = # of wounds.

Results

Patient Profile

Forty-nine patients with 95 wounds were entered into the initial study (Table 4). Four patients with 13 wounds were excluded after initiation of PDWHF therapy because they concomitantly underwent a surgical cure *via* amputation, skin graft, or primary closure. Four patients with 11 wounds were further excluded because they were lost to follow-up. Thus, the final analysis includes 41 patients with 71 wounds. In this analysis, the statistics are weighted, that is to say, a patient with one wound contributed no less statistical significance than a patient with four wounds.

The age of the patients ranged from 11 to 80 years, with the highest number of patients in the 41–70 year old age bracket. When broken down by diagnosis, patients with venous stasis and arterial insufficiency were the eldest, and patients with diabetes, diabetes with renal transplants, and decubitus ulcers were the youngest.

The wound frequency per patient was greatest in the "other" (trauma, vasculitis, evacuated abscess, etc.) diagnostic category, with 23 wounds in eight patients. Most diagnosis groups had an average of two wounds per patient, while the group of transplanted diabetics averaged only one wound per patient.

Wound Severity Score

The wound severity scores had a possible range of 0–97. The mean scores with their standard deviations are presented below. At initial evaluation, the average total wound score (TWS) was 21.6 ± 11.2. When analyzed by diagnosis group, the patients with venous stasis ulcers had the worst average score (30 ± 13.9) followed by those with arterial insufficiency (24.2 ± 7.8), diabetes (22.1 ± 13.0),

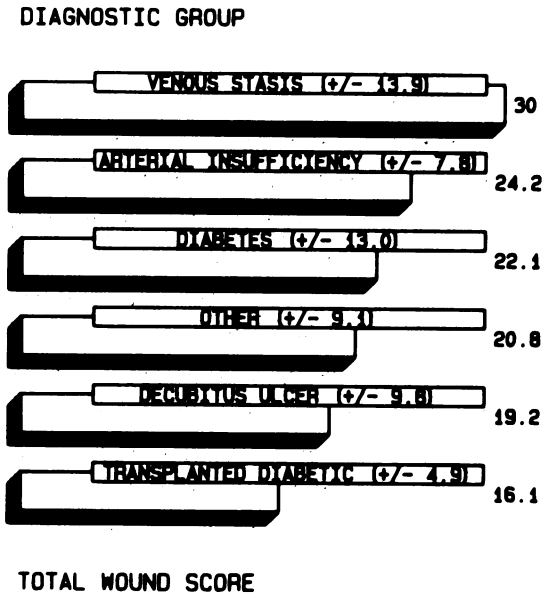

FIG. 1. Initial total wound score by diagnosis grouping. Possible range: 0–97; total wound score: 21.6 ± 11.2.

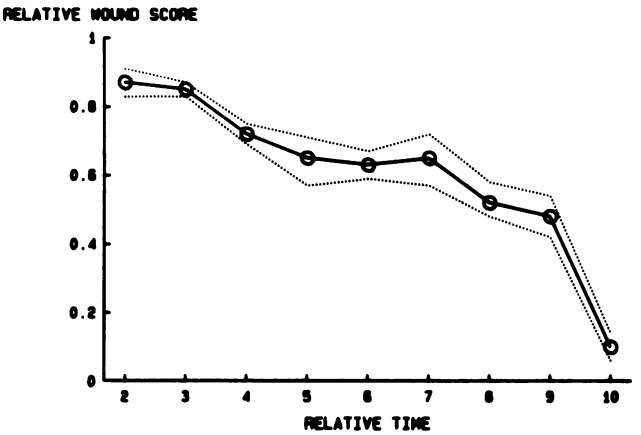

FIG. 2. To compare the change in wound score over time, the relative wound score for each patient was compared to the relative time to 100% healing. The change in wound score was similar in all patients. Mean relative wound score (Θ); ± standard deviation (· · · · ·). N = 31 patients; relative time = weeks/weeks to total healing; relative wound score = wound score/initial wound score.

other (20.8 ± 9.1), decubitus ulcers (19.2 ± 9.8), and diabetes with renal transplants (16.1 ± 4.9) (Fig. 1).

An infection score was determined by adding the periwound erythema score, periwound edema score, and wound purulence score. The worst possible score was 14 and the average infection score for all groups at initial evaluation was 3.0 ± 3.3. When analyzed by diagnosis group, patients with venous stasis ulcers averaged 7.0 ± 3.4, followed by those with arterial insufficiency (6.3 ± 4.4), diabetes with renal transplants (3.2 ± 3.2), other (3.1 ± 2.4), diabetes (2.2 ± 3.1), and decubitus ulcers (1.4 ± 2.6).

Size and wound duration scores were also worst in the venous stasis group. Transplanted diabetic patients had the smallest average wound size and wound duration scores.

To compare the progression of wound score over time among patients, the relative wound score was plotted *versus* the relative time to 100% healing for 31 patients (Fig. 2). The relative wound score showed a gradual decrease over time, with a sharp decrease toward the end of treatment when the wound was almost healed.

PDWHF Stimulated Healing Rates

Healing rates were determined as the time in weeks to achieve 50, 80, and 100% epithelization (T50, T80, and T100, respectively). The T2 represents the time in weeks to 100% epithelization after the initiation of PDWHF therapy.

The overall healing rates are found in Table 5 and are reiterated below with their standard deviations. The average time to 50% healing was 4.5 ± 3.6 weeks, the average time to 80% healing was 7.1 ± 5.1 weeks, and the average time to 100% healing was 10.6 ± 6.1 weeks. The time to 100% healing after initiation of PDWHF therapy was 7.5 ± 6.5 weeks.

The effect of the wound location on PDWHF-stimulated healing was determined. Regardless of the wound location, the mean times to 50, 80, and 100% healing are similar and not statistically different (Fig. 3).

The effect of age on PDWHF stimulated healing was also analyzed by grouping the patients into three relatively numerically equal categories: 0–45 years, 46–60 years, and 61+ years. There was no statistically significant difference in mean healing times based on these groupings (Fig. 4).

The initial six patient diagnostic categories were screened for overall healing rates. The initial observation revealed that the mean healing times to the designated endpoints differed only for those patients who were classified as transplanted diabetics or arterially insufficient. The remaining four groups were thus consolidated into the final "other" category. In the final analysis, the diagnosis of arterial insufficiency carried a statistically sig-

TABLE 5. *Overall Mean Patient Healing Times*

	Mean Time in Weeks	Standard Deviation	95% Confidence Limit
Time to 50% healing	4.53	3.58	1.12
Time to 80% healing	7.15	5.09	1.59
Time to 100% healing	10.63	6.10	1.91
T100 from initiation of PDWHF Rx	7.47	6.53	2.18

T100 *versus* T2, t = 2.26, p = 0.025.

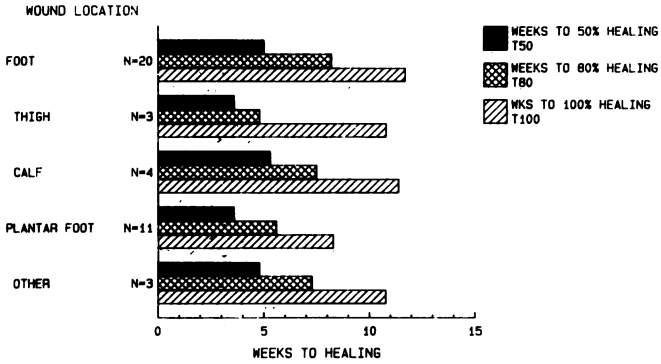

FIG. 3. The effect of wound location on PDWHF stimulated wound healing. Location had no statistically significant effect on the rate of PDWHF stimulated repair.

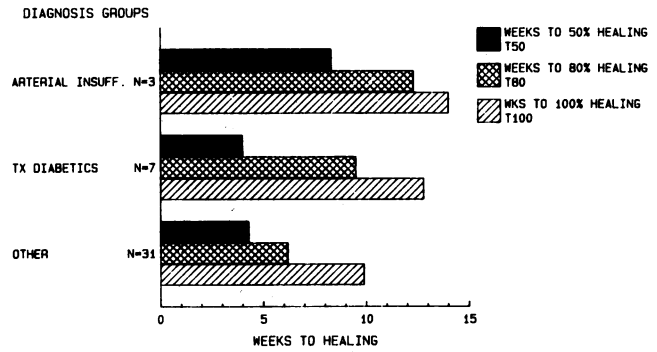

FIG. 5. The effect of diagnosis on PDWHF stimulated wound healing. "Other" diagnosis = diabetes, venous stasis, trauma, decubitus, etc. Patients with arterial insufficiency had a statistically significant delay in total healing time when compared to the "other" category. Otherwise, there was no significant difference between diagnosis groups. Arterial insufficiency *vs.* Tx diabetics, t = 0.35, p = 0.40; arterial insufficiency *vs.* other, t = 1.83, p = 0.05; Tx diabetics *vs.* other, t = 1.00, p = 20.

nificantly longer mean 100% healing time when compared to the "other" category only. There were no other recognized differences between any other diagnostic subsets (Fig. 5).

The timing of PDWHF initiation, however, did have a significant effect on the T50, T80, and T100. Seventeen patiets had immediate PDWHF therapy at their first visit, while 24 patients had delayed PDWHF therapy. At all three healing benchmarks, the patients who received immediate PDWHF therapy healed faster than those who had delayed PDWHF therapy (Table 6).

Recidivism Rates

Eleven percent (4/41) of the patients and 7% (5/71) of the epithelized wounds broke down during the immediate follow-up period. All of these wounds healed with subsequent PDWHF therapy.

Complications of PDWHF Therapy

There was no abnormal tissue formation, keloid, or hypertrophic scarring observed. In addition, there were no infectious complications directly attributable to the application of the PDWHF.

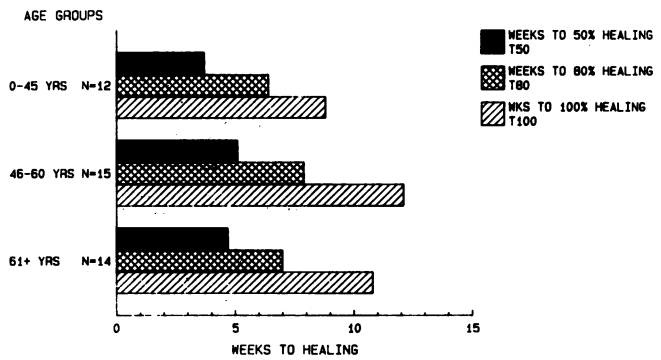

FIG. 4. The effect of age on PDWHF stimulated wound healing. Age had no statistically significant effect on PDWHF stimulated wound repair. 0–45 *vs.* 46–60, t = 1.48, p = 0.10; 0–45 *vs.* 61+, t = 0.95, p = 0.20; 45–60 *vs.* 61+, t = 0.54, p = 0.30.

Multivariant Analysis

A multiple regression analysis of the various components of the wound scores, wound areas, and delay *versus* no delay in PDWHF therapy showed that only the initial size score and the timing of PDWHF therapy had statistically significant correlations to 100% epithelization (Table 7). When a regression analysis of the initial size score *versus* time to 100% healing was calculated, a correlation of 0.66 with p = 0.005 was obtained. The regression equation relating these two variables is: $y = 0.66 + 0.89 \times$ size score + 4.42a (where a = 0 if treatment was started at the

TABLE 6. *Effect of Immediate* Versus *Delayed Treatment with PDWHF on Wound Healing**

	Number of Patients	Mean Healing Time	Standard Deviation	p Value
T50 immediate PDWHF treatment	17	3.0	2.6	0.012
T50 delayed PDWHF treatment	24	5.6	3.8	
T80 immediate PDWHF treatment	17	4.2	3.2	0.001
T80 delayed PDWHF treatment	24	9.1	5.2	
T100 immediate PDWHF treatment	17	6.9	4.1	<0.001
T100 delayed PDWHF treatment	24	13.1	6.0	

* At all measured points, those patients where PDWHF was delayed took longer to heal.

TABLE 7. *Multiple Regression Analysis of Wound Variables Versus Time to 100% Healing*

	r Value	p Value
Initial size score	0.57	<0.001
Delay *versus* no delay in PDWHF Rx	0.66	0.009
Initial wound area	0.67	0.392
Erythema score	0.67	0.538
Edema score	0.68	0.410
Fibrin score	0.69	0.486
Brawny edema score	0.69	0.500
Pitting edema score	0.69	0.645
Wound duration score	0.69	0.799
Purulence score	0.69	0.794
Initial wound score	0.67	0.471

initial evaluation and a = 1 if treatment was delayed) (Fig. 6).

Pearson correlation coefficients were calculated and are presented in Table 8. At T50 only the initial size score and the size score when PDWHF therapy was initiated are highly statistically significant. At T100 the highest correlation was with the initial size score, the total wound score when PDWHF was started, and the size score when PDWHF was started. One negative correlation was found. At T50 the presence of pitting limb edema gave an r of −0.1 but the p = 0.27.

No other single component of the derived wound score, age, duration, nor anatomic distribution was shown to correlate statistically to the mean healing times for the wounds treated with PDWHF in this study.

Discussion

The process of wound repair involves a complex interaction among biochemical amplification cascades, circulating platelets and monocytes, locally acting growth factors, and skin and connective tissue cells. The mechanical process of wounding causes tissue disruption that fractures tissue capillaries, causing activation of Hageman factor (XII) and platelets.[1] Activation of Hageman factor in turn activates the clotting, complement, plasminogen, and kinin cascades.[5] The clotting cascade generates thrombin, which stimulates platelet release of locally acting growth factors; the complement cascade generates C5a, which is a chemoattractant for neutrophils and monocytes; plasminogen activation generates plasmin, which degrades fibrin; and kinin activation produces bradykinin, which causes microvascular vasodilation at the wound edge.

The locally acting growth factors from thrombin-activated platelets initiate the connective tissue response by causing division and migration of fibroblasts and formation of new capillaries.[2] Circulating monocytes become wound macrophages as they migrate into the wound space. The hypoxic wound environment is a potent stimulus for macrophage-derived angiogenesis factor production[6,7]; in

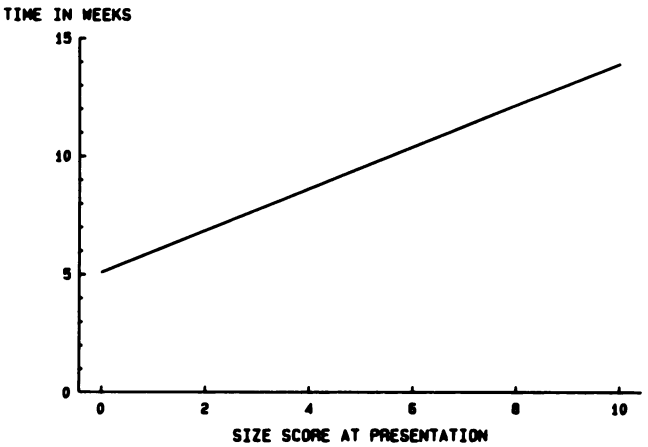

FIG. 6. Multiple regression analysis of the initial size score at presentation *versus* time to 100% healing. This correlation (r = 0.66) is highly significant (p = 0.005). T100 Y = 0.66 + 0.89 (size score) + 4.42a (a = 0; if Rx was delayed, a = 1).

addition, other environmental or biochemical signals stimulate production of macrophage-derived growth factors. These two factors continue to stimulate fibroblast migration, division, and enhanced structural macromolecule synthesis. The angiogenesis factor from wound macrophages continues to stimulate the neovascularization that was initiated by platelets.

The end result of this complex interaction is transformation of a resting connective tissue into an area of intense cellular movement, division, and biosynthesis, which results in closure of the wound space with a neovascularized collagen mesh.

Granulation tissue formation is followed by epidermal division and migration, which covers the collagen–vascular mesh with new skin.

Recent advances in locally acting growth factor biology and technology have more fully delineated the role of these potent biochemicals in the healing process. In wound

TABLE 8. *Pearson Correlation Coefficients (r Value/p Value)*

	T50	T80	T100	T2
Initial size score	0.43/0.002	0.46/0.001	0.57/0.001	0.46/0.001
Pedal pulse score	0.18/0.125	0.33/0.016	0.31/0.023	0.20/0.100
Initial total wound score	0.20/0.13	0.29/0.03	0.44/0.002	0.33/0.013
Total wound score at PDWHF Rx	0.27/0.045	0.37/0.008	0.53/0.001	0.41/0.003
Initial wound area	0.29/0.032	0.34/0.014	0.43/0.003	0.29/0.028
Wound area at PDWHF Rx	0.27/0.046	0.32/0.021	0.42/0.003	0.34/0.011
Size score at PDWHF Rx	0.43/0.002	0.46/0.001	0.56/0.001	0.42/0.002

T100, T80, T50 = time to 100%, 80%, 50% healing.
T2 = time to total healing from PDWHF treatment.
Limb edema at T50 r = −0.1 but p = 0.27.

repair the two sources of these factors are the platelet and the wound macrophage. Platelets release platelet-derived growth factor (PDGF), platelet-derived angiogenesis factor (PDAF), a platelet-derived epidermal growth factor, and platelet factor 4. PDGF is a potent fibroblast mitogen and chemoattractant, which when purified has no endothelial cell chemoattractant activity.[8-10] PDAF causes new capillary formation from the existing microvasculature.[2] A platelet-derived epidermal growth factor has been described but is poorly characterized. Platelet factor 4 is a chemoattractant for neutrophils. These four factors initiate the connective tissue response that results from injury.

The wound macrophage takes over production of these factors from the platelet and the process of repair continues until the wound is healed. Wound macrophages produce a growth factor very similar in biochemical and cellular activity to PDGF.[11] They also produce an angiogenesis factor similar to PDAF.[4] To date, there is no epidermal growth factor activity found in the wound macrophage.

Chronic nonhealing wounds or cutaneous ulcers are the result of inadequate repair. A complete delineation of all the causes of inadequate repair is beyond the scope of this discussion. The major common etiology of cutaneous ulcers is decreased skin perfusion and infection. Decreased skin perfusion can result from large artery stenosis or occlusion as in atherosclerosis, medium size artery stenosis or occlusion as in diabetic atherosclerosis, small artery disease as in vasculitis, or capillary dysfunction as in diabetic basement membrane thickening. Venous hypertension from postphlebitis syndrome, extensive tissue trauma, or pressure also causes decreased skin, subcutaneous, and occasionally bone perfusion. The end result is tissue ischemia and/or death.

Infection is also a common occurrence in cutaneous ulcers. The process of host defense against infection consumes oxygen. In a marginally perfused tissue, host defense activities against infection can consume so much oxygen that skin, connective tissue, and bone viability is compromised.

Traditional treatment of these nonhealing ulcers has consisted of a passive attempt to alter the local environment to favor repair over further tissue loss. Topical, oral, or parenteral antibiotics are administered to decrease the bacterial count in the wound, protective dressings that may alter the wound macroenvironment are used to decrease tissue trauma and augment repair, and various topical agents that chemically debride the wound, remove wound exudate, or change the wound environment are attempted. These passive treatments can result in eventual repair of the wound as the destructive forces that favor tissue death are slowly replaced by reparative tissue growth.

The chronicity and poor results with conventional therapy are reflected in our patient pool. The average duration of conventional therapy in our patients was 198 weeks (range: 1–1820 weeks).

These factors direct cellular movement, division, and synthetic activity. Topical application should transform a chronically nonhealing wound that has little cellular activity into a healing wound with a maximal cellular reparative response. To date, the lack of availability of these locally acting growth factors has precluded their clinical use.

Previously published data showed that platelets release all the locally active growth factors necessary to initiate wound repair. The data presented in this manuscript demonstrate, for the first time, that the topical application of PDWHF stimulates repair of chronically nonhealing human wounds resulting in accelerated granulation tissue formation and epithelization. PDWHF therapy is safe, efficacious, and cost effective. There was no evidence of overhealing such as hypertrophic scar or keloid formation. The patients applied the PDWHF at home without difficulty, requiring only periodic outpatient examination. All wounds that recurred were subsequently healed with additional PDWHF treatment.

The development of a wound severity scoring system based on clinical observation and simple measurements allows categorization of the wounds into severity groups for comparison and a means of numerically following the clinical course of wound repair. Measuring these wound, patient, and anatomical parameters to generate the wound score allowed a statistical analysis of the correlation of these traditional variables with measured wound repair rates that were stimulated by PDWHF. The result of these correlations demonstrates the utility of using the locally acting growth factor signals that control cellular function. The only parameters that had significant correlation on multiple regression analysis were the initial wound size and the timing of the initiation of PDWHF therapy. The presence of infection, exposed bone or tendon, wound location, patient age, patient diagnosis (other than arterial insufficiency), or wound duration had no correlation with the rate of repair. This analysis suggests that the PDWHF treatment can stimulate reparative cellular response in many different clinical situations. It does not prove that our traditional clinical treatment program of revascularization, debridement, and antibiotics is no longer needed. We cannot determine, from these data, whether the rate of PDWHF stimulated repair would have been decreased had these adjunctive treatments not been carried out. To prove the relative importance of PDWHF therapy *versus* standard therapy, a blinded, prospective trial is required.

In summary, this study demonstrates for the first time that locally acting growth factors obtained from human platelets and applied topically to chronic nonhealing human ulcers stimulates granulation tissue formation and accelerated epithelization. The rate of wound repair stimulated by PDWHF correlates only with the initial wound

size and the timing of PDWHF administration. The treatment is safe, efficacious, and cost effective.

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Discussion

DR. THOMAS K. HUNT (San Francisco, California): It has been 3 or 4 years since Dr. Knighton presented the first paper on platelets and wound healing before this Society. I might add that the prediction of that paper, that platelets would be found to play an important part of the wound healing mechanism, has now been reinforced experimentally by several other investigators, and I am gratified that human trials have progressed this far in such a short period.

Experimental and clinical observations are uniform in recognizing that various so-called growth factors have salutary benefits on wounds of any persuasion—new, old, chronic, or whatever. In this sense Dr. Knighton's results reinforce in humans what we already know in animals. However, this study should drive yet another nail in the coffin of the old concept that mother nature allows no tampering with wound healing. Sadly, I suspect a few more nails will be necessary before medical opinion finally gives way.

The paper is unique in that humans are studied and that a severity score based on factors in addition to size is proposed. This score has allowed a statistical approach that is unique in this field. On the other hand, the study is not unique in that the authors have found, like almost everyone before them, that it is very difficult to study a single variable in wound healing, and in fact they have a multivariable study. It remains to be seen, I think, whether the statistical approach engendered by the score can overcome (as it has from time to time in other areas) the value of a simple, direct method to test single variables, *i.e.*, a prospective test system in a standardized wound.

I suspect that some of the other discussants will find some fault with this paper. The power of platelet factor is not fully demonstrated; the scoring system is not fully validated yet; but this is a good start, and I submit that it would be very difficult to present a paper on a scoring system alone before a Society such as this. Most important, here, is the use of historical controls only. Today, this is not acceptable, but I remind the Society that data generated in just that way has led to many important discoveries.

The paper opens several new areas, and I confidently expect that it will be the start of a new generation of human studies. There is no doubt in my mind that it will lead to something useful. In that regard I would like to ask a couple of questions:

First, why do the patients who have delayed start of therapy do better? I would have thought that each would have done the same, starting at any point in their history. Why do bone defects not make more of a problem? I should have thought that the rigid, bony edges that do not allow collapse of wounds would have led to a longer time for recovery in both groups. I hope that perhaps in your closing remarks you will give us some details about how you prepared the factor.

DR. STANLEY M. LEVENSON (Bronx, New York): I had the opportunity to review this paper last night and enjoyed it very much. I want to compliment Dr. Knighton and his colleagues for attempting to study this important problem in patients. I am glad to see that the two nurses who did so much of the work are coauthors of the paper.

I would like to read the avowed aim of the study: "To test the efficacy of topically applied platelet derived wound healing factors in stimulating repair of chronic, non-healing cutaneous wounds."

Dr. Knighton has described a very simple technique for the preparation of the platelet derived growth factor(s), and you will notice that he made no attempt to purify any of the factors; and that is good, because by preparing the autologous platelet preparation the way he did it likely contained all, supposedly, of the active growth promoting components. There were no complications associated with the use of the platelet preparation, and apparently there was a dramatic clinical effect. That sounds great!

On the other hand, was the therapeutic effect due to the platelet preparation? The experimental design of the study really does not lend itself to that conclusion. That does not mean that the therapeutic effect was not due to the platelet derived growth factor(s) preparation, but that study as designed does not prove that, because there were no appropriate controls in the study. I realize that it is not easy to do a double-blind study, but I think that should have been done. Whether Dr. Knighton will feel that to initiate such a study now is morally justified in view of his findings and interpretation is something he will have to decide.

I want to ask Dr. Knighton a couple of questions about the dressing technique. He mentioned that the test material was applied for 12 hours during the day and then removed and a silver sulfadiazine ointment dressing applied. Was that done because he wanted to have the chemotherapeutic effect of a local antibiotic or chemotherapeutic agent? Did he want to save the platelet derived growth factor preparation because of shortage of supply? Did he feel that the alternating treatment had some not clearly defined mechanistic or mechanical effect? Also, was there any special reason why 12-hour shifts were used for the platelet preparation and silver sulfadiazine ointment? That is not a facetious question, because we all know that depending on when something is done during the 24 hours of a day (diurnal rhythm) there may be a dramatically different effect. I need only remind you that a dose of endotoxin that will be 100% lethal for mice when given at 2 A.M. is nonlethal when given at 10 A.M.

Also, the acceleration of healing ascribed for the more rapid wound closure was accelerated epithelialization, but, as demonstrated in the pictures of the case presented, closure of the wound was chiefly by contraction, and that is what I would have anticipated. If one goes back to the studies of Alexis Carrel about 70 years ago dealing with the healing of open wounds, he pointed out that contraction, contracture, and ep-